abcam

Product datasheet

Anti-MEK3 antibody [EPR17345-104] ab195037

KO VALIDATED Recombinant RabMAb

6 References 13 Images

Overview

Product name	Anti-MEK3 antibody [EPR17345-104]
Description	Rabbit monoclonal [EPR17345-104] to MEK3
Host species	Rabbit
Tested applications	**Suitable for:** IHC-P, WB, ICC/IF, IP, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HepG2, HeLa and MCF7 whole cell lysates; Human skeletal muscle, mouse spleen and rat spleen lysates. IHC-P: Human skeletal muscle, Human lung adenocarcinoma, mouse skeletal muscle and rat skeletal muscle tissues. ICC/IF: NIH/3T3 cells. IP: HeLa whole cell extract.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR17345-104

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab195037 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/5000. Detects a band of approximately 39, 34 kDa (predicted molecular weight: 39 kDa).
ICC/IF		1/250.
IP		1/90.
Flow Cyt (Intra)		1/1000. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Target

Function Dual specificity kinase. Is activated by cytokines and environmental stress in vivo. Catalyzes the concomitant phosphorylation of a threonine and a tyrosine residue in the MAP kinase p38.

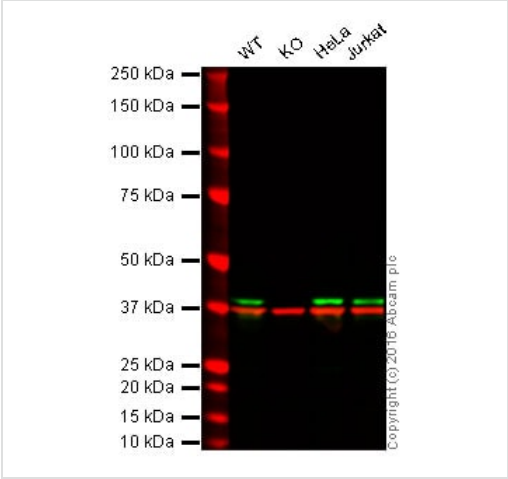
Tissue specificity Abundant expression is seen in the skeletal muscle. It is also widely expressed in other tissues.

Involvement in disease Note=Defects in MAP2K3 may be involved in colon cancer.

Sequence similarities Belongs to the protein kinase superfamily. STE Ser/Thr protein kinase family. MAP kinase kinase subfamily.
Contains 1 protein kinase domain.

Post-translational modifications Autophosphorylated.
Phosphorylation on Ser-218 and Thr-222 by MAP kinase kinase kinases regulates positively the kinase activity.
Yersinia yopJ may acetylate Ser/Thr residues, preventing phosphorylation and activation, thus blocking the MAPK signaling pathway.

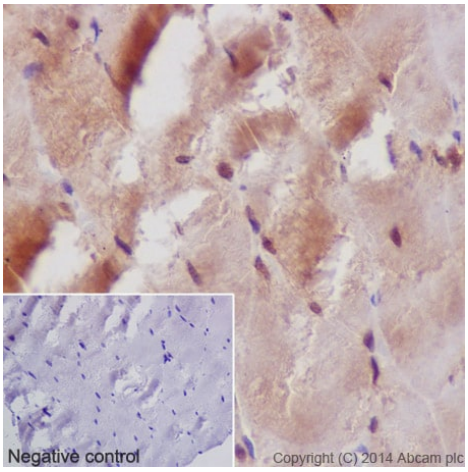
Images



Western blot - Anti-MEK3 antibody [EPR17345-104] (ab195037)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: MEK3 knockout HAP1 cell lysate (20 µg)
Lane 3: HeLa cell lysate (20 µg)
Lane 4: Jurkat cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab195037 observed at 40 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab195037 was shown to specifically react with MEK3 when MEK3 knockout samples were used. Wild-type and MEK3 knockout samples were subjected to SDS-PAGE. ab195037 and **ab8245** (loading control to GAPDH) were both diluted to 1/5000 and 1/10000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



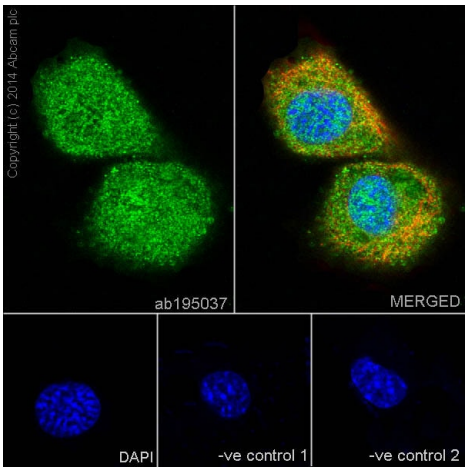
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MEK3 antibody [EPR17345-104] (ab195037)

Immunohistochemical analysis of paraffin-embedded Human skeletal muscle tissue labeling MEK3 with ab195037 at 1/500 dilution followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) secondary at 1/500 dilution. Cytoplasmic and nucleus staining on Human skeletal muscle is observed. Counter stained with Hematoxylin.

Negative control: Using PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

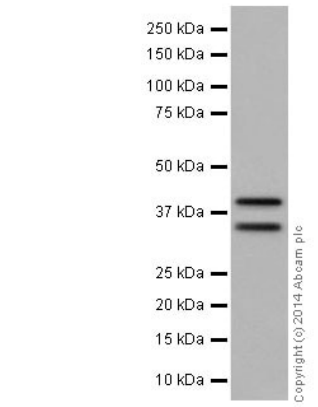
1:Ben-Levy R,et.al.(1998) Nuclear export of the stress-activated protein kinase p38 mediated by its substrate MAPKAP kinase-2. Curr Biol, 8(19):1049-1057.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-MEK3 antibody [EPR17345-104] (ab195037)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (Mouse embyro fibroblast cells) cells labeling MEK3 with ab195037 at 1/250 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/500 dilution (green). Confocal image showing both nuclear and cytoplasmic staining on NIH/3T3 cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).
The negative controls are as follows:
-ve control 1: ab195037 at 1/250 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
-ve control 2: **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/500 dilution.



Western blot - Anti-MEK3 antibody [EPR17345-104] (ab195037)

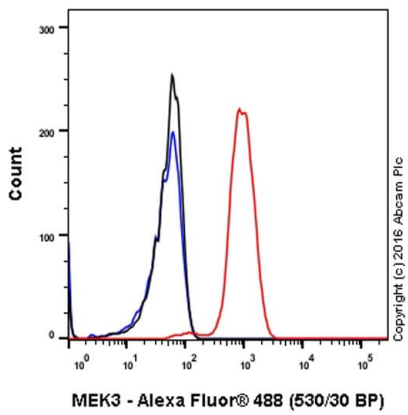
Anti-MEK3 antibody [EPR17345-104] (ab195037) at 1/50000 dilution + HepG2 (Human liver hepatocellular carcinoma) whole cell lysates at 10 µg

Secondary
Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Predicted band size: 39 kDa
Observed band size: 34,39 kDa

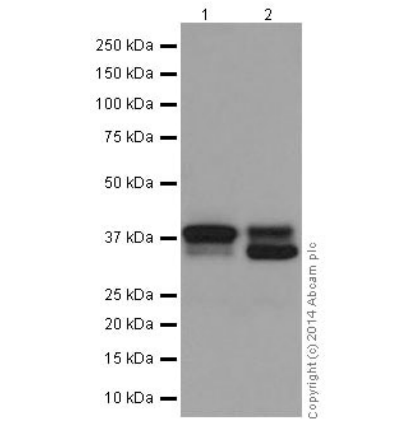
Blocking/Dilution buffer: 5% NFDM/TBST.

Based on the sequence analysis, ab195037 recognizes 3 isoforms of MEK3 ranging from 36kDa to 40KDa. The multi-bands should be MEK3 isoforms.



Flow Cytometry (Intracellular) - Anti-MEK3 antibody [EPR17345-104] (ab195037)

Intracellular Flow Cytometry analysis of NIH/3T3 (mouse embryo) labelling MEK3 with purified ab195037 at 1/1000 (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. Alexa Fluor® 488 goat anti-rabbit IgG (1/2000) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



Western blot - Anti-MEK3 antibody [EPR17345-104] (ab195037)

All lanes : Anti-MEK3 antibody [EPR17345-104] (ab195037) at 1/10000 dilution

Lane 1 : HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysates
Lane 2 : MCF7 (Human breast adenocarcinoma cell line) whole cell lysates

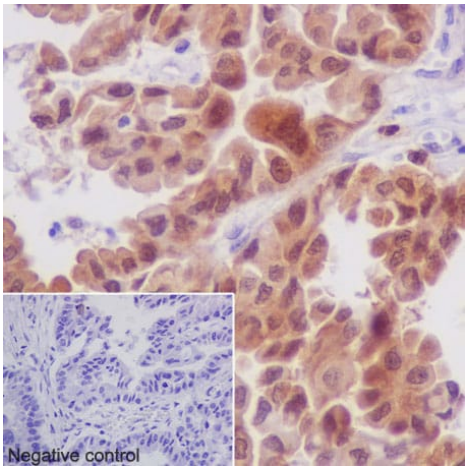
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Predicted band size: 39 kDa
Observed band size: 34,39 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Based on the sequence analysis, ab195037 recognizes 3 isoforms of MEK3 ranging from 36kDa to 40KDa. The multi-bands should be MEK3 isoforms.

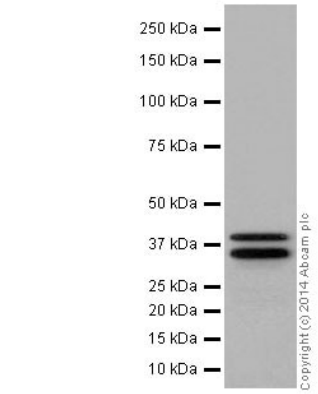


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MEK3 antibody [EPR17345-104] (ab195037)

Immunohistochemical analysis of paraffin-embedded Human lung adenocarcinoma tissue labeling MEK3 with ab195037 at 1/500 dilution followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) secondary at 1/500 dilution. Cytoplasmic and nuclear staining on cancer cells of Human lung adenocarcinoma is observed. Counter stained with Hematoxylin.

Negative control: Using PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-MEK3 antibody [EPR17345-104] (ab195037)

Anti-MEK3 antibody [EPR17345-104] (ab195037) at 1/5000 dilution + Human skeletal muscle lysates at 10 µg

Secondary

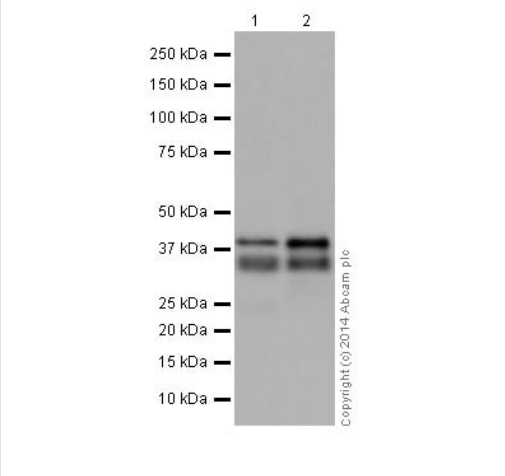
Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 39 kDa

Observed band size: 34,39 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Based on the sequence analysis, ab195037 recognizes 3 isoforms of MEK3 ranging from 36kDa to 40KDa. The multi-bands should be MEK3 isoforms.



Western blot - Anti-MEK3 antibody [EPR17345-104] (ab195037)

All lanes : Anti-MEK3 antibody [EPR17345-104] (ab195037) at 1/10000 dilution

Lane 1 : Mouse spleen lysates
Lane 2 : Rat spleen lysates

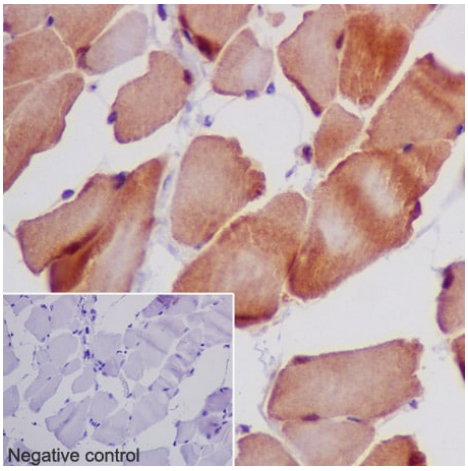
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 39 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Based on the sequence analysis, ab195037 recognizes 3 isoforms of MEK3 ranging from 36kDa to 40KDa. The multi-bands should be MEK3 isoforms.

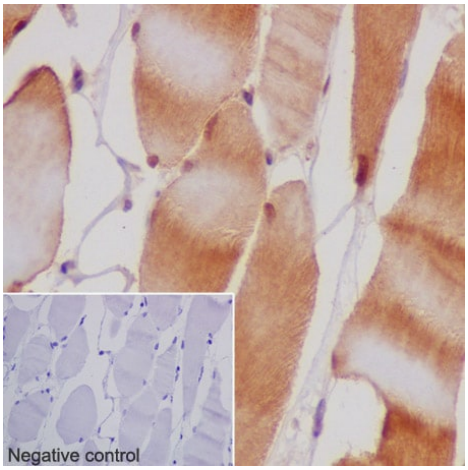


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MEK3 antibody [EPR17345-104] (ab195037)

Immunohistochemical analysis of paraffin-embedded Mouse skeletal muscle tissue labeling MEK3 with ab195037 at 1/500 dilution followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) secondary at 1/500 dilution. Cytoplasmic and nuclear staining on mouse skeletal muscle is observed. Counter stained with Hematoxylin.

Negative control: Using PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

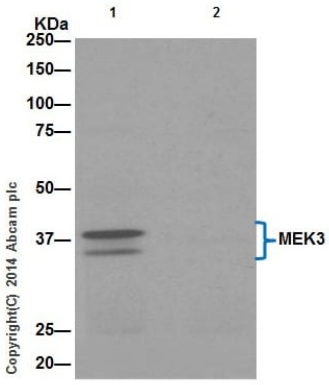


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MEK3 antibody [EPR17345-104] (ab195037)

Immunohistochemical analysis of paraffin-embedded Rat skeletal muscle tissue labeling MEK3 with ab195037 at 1/500 dilution followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) secondary at 1/500 dilution. Cytoplasmic and nuclear staining on rat skeletal muscle is observed. Counter stained with Hematoxylin.

Negative control: Using PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunoprecipitation - Anti-MEK3 antibody [EPR17345-104] (ab195037)

MEK3 was immunoprecipitated from 1mg of HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell extract with ab195037 at 1/90 dilution. Western blot was performed from the immunoprecipitate using ab195037 at 1/1000 dilution. Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG, was used as secondary antibody at 1/1500 dilution. Lane 1: HeLa whole cell extract. Lane 2: PBS instead of HeLa whole cell extract.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Based on the sequence analysis, ab195037 recognizes 3 isoforms of MEK3 ranging from 36kDa to 40KDa. The multi-bands should be MEK3 isoforms.

Anti-MEK3 antibody [EPR17345-104] (ab195037)

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors